Prognostic impact of anticardiolipin antibodies in women with recurrent miscarriage negative for the lupus anticoagulant

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BACKGROUND: Anticardiolipin antibodies (ACA) are found with increased prevalence in women with unexplained recurrent miscarriage (RM) but their impact on future pregnancy outcome in lupus anticoagulant (LAC) negative patients needs better quantification. METHODS: The impact of a repeatedly positive ACA test on the chance of live birth in the next pregnancy after adjustment for relevant prognostic factors was studied in 147 RM patients who had been included in placebo-controlled trials of immunotherapy. Patients with LAC were excluded and none of the patients received therapy with anticoagulation or prednisone. RESULTS: 60/147 patients (41%) were repeatedly ACA positive according to cut-off values derived from this study. The adjusted odds ratio (OR) for live birth among ACA positive patients was 0.36 (95% CI 0.2–0.7, P = 0.006). Using cut-off values derived from a normal population, the adjusted OR for live birth among ACA positive patients was 0.48 (95% CI 0.2–1.1, P = 0.10). Positivity for IgM ACA was found to be much stronger correlated to pregnancy outcome than IgG ACA. CONCLUSIONS: In RM women not receiving anticoagulation or prednisone, the presence of ACA in the absence of LAC most likely reduces the chance of live birth by 36–48% compared with the absence of both ACA and LAC. This reduction is inferior to what has been reported from studies where no adjustments for prognostic variables were undertaken and LAC positive patients were included.

Key words: anticardiolipin antibodies/immunotherapy in placebo-controlled trials/logistic regression analysis/prognostic variables/recurrent miscarriages

Introduction

During the last two decades much attention has been drawn to the relationship between autoantibodies and pregnancy loss (Cowshock et al., 1986; Petri et al., 1987; Unander et al., 1987; Maier and Parke, 1989; Christiansen et al., 1989, Out et al., 1992; Rai et al., 1995a,b; Simpson et al., 1998). Most focus has been on antiphospholipid antibodies (APA), which are autoantibodies directed against plasma proteins bound to suitable anionic (not necessarily phospholipid) surfaces in the inner layer of biological membranes (Galli and Barbui, 2003). It is generally believed that the clinically relevant APA bind to proteins with affinity for phospholipids, the most important being β2-glycoprotein I (β2GPI). Among APA, the majority of studies have focused on anticardiolipin antibodies (ACA) and lupus anticoagulant (LAC). ACA are measured in an enzyme-linked immunosorbent assay (ELISA) test whereas the activity of LAC is measured by phospholipid coagulation assays (Harris, 1995). However, the presence of ACA and LAC is found to have a low concor-
the prospective studies have been recruited from very different populations: women with systemic lupus erythematosus (Ginsberg et al., 1992; Out et al., 1992), healthy women with low risk pregnancies (Lockwood et al., 1989; Infante-Rivard et al., 1991; Pattison et al., 1993; Lynch et al., 1994; Yasuda et al., 1995), women with recurrent miscarriage (RM) (Barbui et al., 1988; Parazzini et al., 1991; Rai et al., 1995b) or mixtures of these populations. In the prospective studies, the effect of ACA on live birth rate has been reported very differently with some studies finding no effect (Taylor et al., 1990; Infante-Rivard et al., 1991; Out et al., 1992; Melk et al., 1995, Simpson et al., 1998), and other studies reporting a great negative impact of these antibodies (Tulppala et al., 1993; Yasuda et al., 1995; Rai et al., 1995b).

The different results in the prospective studies could partly be attributed to poorly standardized ACA tests (Harris et al., 1987b) reflected in a significant interlaboratory variation in the detection of ACA (Peaceman et al., 1992; Roberts et al., 2002), significant fluctuations of the antibody levels over time in the same patient (Rai et al. 1995a; Donohoe et al. 2002) and very different cut off levels (Quenby and Farquharson, 1993; Tulppala et al., 1993; Rai et al., 1995 a,b). Another important reason for the different estimates of risk conferred by ACA may be that the patient populations monitored have been very heterogeneous with regard to underlying disease, the number of miscarriages, age and other important prognostic factors. Few authors have adjusted for the influence of other prognostic variables by using logistic regression analysis (Lockwood et al., 1989; Infante-Rivard et al., 1991; Out et al. 1992, Lynch et al., 1994). In neither of the studies did the included patients suffer from RM. Finally, very few of the published prospective studies have focused especially on RM patients with exclusively ACA (Tripllet, 1989), in most prospective studies ACA and LAC positive patients have been considered together.

For a clinician who is treating patients with RM, previous studies do not adequately answer the question: what is the prognostic value of an elevated ACA titre in the absence of LAC in non-anticoagulation treated RM patients with regard to live birth rate and the development of late pregnancy complications such as preeclampsia, low birth weight and preterm labour? This is the question we try to answer in this study.

Materials and methods

Patients

Over a period of 14 years, 147 patients with RM who had suffered at least three consecutive miscarriages were admitted to our clinic to participate in one of three placebo-controlled studies on the efficacy of allogenic leukocyte immunization (ALT) or intravenous immunoglobulin (Ivlg) treatment in preventing miscarriage (Christiansen et al., 1992, 1995, 2002). A miscarriage was defined as embryonic/fetal death occurring before the end of gestational week 28. The patients had proved normal after investigations for uterine abnormalities (by hysterosalpingography or hysteroscopy), mid-luteal serum progesterone measurement (normal >20 nmol/l), serum thyroxin measurement, and karyotyping. They had all regular menstrual cycles between 25 and 35 days. In the husbands, karyotyping was found to be normal.

In the ALT trial, exclusion criteria were positivity for lymphocytotoxic antibodies, LAC, or high titre of antinuclear antibodies (ANA) or anti-double-stranded deoxyribonucleic acid (α-dsDNA) antibodies.

The Ivlg trials comprised two trials where, in the first trial, patients with at least three previous miscarriages and mainly secondary RM were included whereas, in the second, only patients with a minimum of four previous miscarriages with both primary and secondary RM were included. For both Ivlg trials no immunological or coagulation-related exclusions were made (Christiansen et al. 2002). Two patients included in the trials had a positive LAC test and were not included in the present analysis.

Eighty-four patients (57%) in the total study population had had primary RM, whereas 63 (43%) had suffered secondary RM having carried at least one pregnancy (live birth/stillbirth) beyond the 28th week of gestation before the sequence of miscarriages. Only 26 (4.1%) of the patients’ 625 pregnancy losses had occurred after gestational week 10 (and before week 22). Table I displays background parameters for the patients. There were statistically significant differences between the ALT trial and Ivlg trial with regard to the number of previous miscarriages, the frequency of secondary RM and the frequency of positivity for ACA, partly due to the different inclusion and exclusion criteria in these trials. However, no significant differences in the

Table I. Background details and outcome data for patients included in the study according to trial and allocation to either treatment or placebo

<table>
<thead>
<tr>
<th></th>
<th>ALT trial</th>
<th>Ivlg Trials</th>
<th>P-value</th>
<th>Treatment</th>
<th>Placebo</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>70</td>
<td>77</td>
<td></td>
<td>82</td>
<td>65</td>
<td></td>
</tr>
<tr>
<td>Median age (range)</td>
<td>29 (21–43)</td>
<td>31 (23–41)</td>
<td>0.000</td>
<td>27 (33%)</td>
<td>23 (35%)</td>
<td>0.3</td>
</tr>
<tr>
<td>4 miscarriages</td>
<td>15 (21%)</td>
<td>39 (51%)</td>
<td>0.000</td>
<td>27 (33%)</td>
<td>23 (42%)</td>
<td>0.6</td>
</tr>
<tr>
<td>≥ 5 miscarriages</td>
<td>14 (20%)</td>
<td>29 (38%)</td>
<td>0.05</td>
<td>28 (34%)</td>
<td>15 (23%)</td>
<td></td>
</tr>
<tr>
<td>Primary RM</td>
<td>46 (66%)</td>
<td>38 (49%)</td>
<td>0.05</td>
<td>52 (63%)</td>
<td>32 (49%)</td>
<td>0.08</td>
</tr>
<tr>
<td>Secondary RM</td>
<td>24 (34%)</td>
<td>39 (51%)</td>
<td>0.05</td>
<td>30 (37%)</td>
<td>33 (51%)</td>
<td></td>
</tr>
<tr>
<td>ACA-NP+</td>
<td>10 (14%)</td>
<td>21 (27%)</td>
<td>0.05</td>
<td>19 (23%)</td>
<td>12 (19%)</td>
<td>0.5</td>
</tr>
<tr>
<td>ACA-RM+</td>
<td>23 (33%)</td>
<td>37 (48%)</td>
<td>0.06</td>
<td>35 (43%)</td>
<td>25 (39%)</td>
<td>0.6</td>
</tr>
<tr>
<td>IgM ACA-RM+</td>
<td>13 (19%)</td>
<td>34 (44%)</td>
<td>0.001</td>
<td>25 (31%)</td>
<td>22 (34%)</td>
<td>0.7</td>
</tr>
<tr>
<td>IgG ACA-RM+</td>
<td>14 (20%)</td>
<td>5 (7%)</td>
<td>0.02</td>
<td>14 (17%)</td>
<td>5 (8%)</td>
<td>0.09</td>
</tr>
<tr>
<td>ANA pos. or α-dsDNA pos.</td>
<td>3 (4%)</td>
<td>7 (9%)</td>
<td>0.25</td>
<td>4 (5%)</td>
<td>6 (9%)</td>
<td>0.3</td>
</tr>
<tr>
<td>Outcome-birth</td>
<td>44 (63%)</td>
<td>37 (48%)</td>
<td>0.07</td>
<td>50 (61%)</td>
<td>31 (48%)</td>
<td>0.1</td>
</tr>
<tr>
<td>Treatment</td>
<td>44 (63%)</td>
<td>38 (49%)</td>
<td>0.1</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

P-values calculated using the χ² test.

For abbreviations: RM, ACA-NP, ACA-RM, IgM ACA-RM, IgG ACA-RM, see Materials and methods (section: Establishment of cut-off points); ANA, antinuclear antibodies; α-dsDNA, anti-double-stranded deoxyribonucleic acid.
frequencies of these parameters were found between the treatment and placebo groups, illustrating that the allocation to the two groups had indeed been random.

Treatment procedures
ALT trial: In 44 patients, third party donor buffycoat (leukocyte enriched blood concentrate) was infused intravenously, or husband’s lymphocytes were injected intravenously and subcutaneously at least twice before pregnancy but no injections were given during pregnancy. In 26 patients (the placebo group) autologous leukocytes were infused according to the same protocol as used for allogeneic leukocytes.

IvIg trial
In the first study IvIg or placebo (albumin) 0.4 g/kg bodyweight was infused from 5th week to 8th week and afterwards every second week until week 34. In the second trial 0.8 g/kg bodyweight was infused weekly from 5th week to 10th week and afterwards every fortnight until week 26.

In neither of the trials did any of the patients receive prednisone or anticoagulation therapy such as heparin or aspirin. No hormonal support (progesterone, hCG) was given during pregnancy and all patients conceived spontaneously.

Laboratory analyses
Screening for LAC was performed by measuring the activated partial thrombin time (APTT) in plasma according to the international consensus from the period the study was done. It recommended APTT as a test that alone or in conjunction with other coagulation tests should be the first step in investigation for LAC (Wilson et al., 1999). Thrombocyte-poor citrated plasma from freshly drawn blood was used for the APTT determination. The APTT measurement was undertaken with the Automated APTT kit (Organon Teknika, NC). One hundred microlitres of plasma was incubated with 100 µl of rabbit brain phospholipids together with micronized silica as activator and a buffer for 5 min at 37°C. One hundred microlitres of 20 mM CaCl₂ was added and the clotting time was measured optically with a Behring Coagulation Timer. As a control, the clotting time of samples from a pool of normal plasma was included in every analysis. If APTT was more than 40 s, a confirmatory test for LAC was performed using commercially available kits (Viper Quick LA-test and Viper Quick LA-check; Organon Technics, Durham, NC).

Details concerning the laboratory tests for ANA and α-dsDNA have been described previously (Christiansen et al., 1992, 2002).

Detection of anticardiolipin antibodies
The first blood sample (sample I) was taken before pregnancy in the ALT trial. In the IvIg trials, sample I was taken as soon as a pregnancy test was positive at the latest 5 days after the first day of the missed period. In both the ALT and IvIg trial, sample II was taken between the 6th and 10th gestational week calculated from the last menstrual period in the patient’s index pregnancy; in the ALT trial this was the first subsequent pregnancy after sample I was taken. Among the ACA positive patients in this study, one patient had a 2 week interval between the samples, five had 4 weeks, and three had 5 weeks, whereas the remaining patients had 6 weeks between the two samples.

Samples from patients and controls were allowed to coagulate. Serum was separated by centrifugation, stored at −80°C, and was kept frozen before use. The ACA analyses were made after the trials were concluded using an ELISA assay. The kit (Varelisa; Elias, Freiburg, Germany) uses the principle of a transferable solid phase. The wells of the ELISA plate are coated with bovine phospholipids complexed with β2-GPI using a non-ethanol based water soluble coating buffer in order to preserve the 3-dimensional structure of the cardiolipin molecule. The tested samples were diluted 1:100 with a phosphate buffer supplemented with 10% bovine serum albumin and 1% sodium azide. The kit fulfills the recommendations of Harris et al. (1987a) and the assays were carried out against an established standard serum from the Rayne Institute, London. Photometric quantification at 492 nm and correction for background activity at 620 nm were undertaken, and for the calculation of unknown samples, mean optical densities were compared with the computerized standard curve. The results for immunoglobulin (Ig)M and (Ig)G class ACA were calibrated in M-phospholipid (MPL)– U/ml and G-phospholipid (GPL)–U/ml, respectively. For each test kit, one control serum and seven standard samples in concentrations ranging from 0 to 60 MPL-U/ml and from 0 to 100 GPL-U/ml were included. All samples were determined in duplicate, and samples 1 and II from the same patients were always assayed on the same ELISA plate to avoid the effect of any inter-assay variation. The intra-assay coefficient of variation in this study was in our laboratory found to be <1% for low values (IgM <2 MPL-U/ml, IgG < 4 GPL-U/ml), 2.5% for mid-range values (IgM 2–7 MPL-U/ml, IgG 4–10 GPL-U/ml) and 4% for high values (IgM ≥ 7 MPL-U/ml and IgG ≥ 10 GPL-U/ml). The inter-assay coefficients of variation for this kit have been reported to be 7.8–10.4% for IgG ACA and 18.6% for IgM ACA (Wong et al., 2004). Coefficients of variation below 20% are considered appropriate (Wong et al., 2004).

Establishment of cut-off levels
The impact of a repeatedly positive ACA value on future pregnancy outcome was estimated using two different sets of cut-off levels. The first set was defined as the upper 95th percentiles of ACA measurements carried out on the same ELISA plates as used in this study in a population of 50 Danish non-pregnant women of fertile age (Christiansen et al., 1992). The normal population-based cut-offs were 7.0 MPL-U/ml and 22.0 GPL-U/ml for IgM and IgG ACA, respectively. If a patient was repeatedly positive for IgM ACA, IgG ACA or both of them according to these cut-off levels she was designated IgM ACA-NP+ (normal population positive), IgG ACA-NP+ or ACA-NP+.

The second set of cut-off values was established as the 10% percentiles (deciles) above which the patients had a significant decline in live birth rate in the present prospective study. Patients being repeatedly positive according to these cut-off levels would be designated IgM ACA-RM+ (recurrent miscarriage population positive), IgG ACA-RM+ or ACA-RM+.

Statistics
Differences of background variables between the treatment and the placebo groups, between the ALT and the IvIg trials and differences between subsequent birth rates and the presence/absence of ACA, were tested with the χ² test.

Heterogeneity of live birth rates between the six different ACA-RM+ subgroups in the study (ACA-RM+ patients in the ALT trial and the first and second IvIg trial allocated to treatment or placebo) was tested by the χ² test. The same heterogeneity test was done for the patients in the six ACA-RM– groups.

The outcome measure of live birth versus miscarriage (odds for live birth) was studied by a stepwise, backward, multivariate logistic regression analysis performed to identify the possible prognostic variables that best predicted the outcome of live birth. Data analyses
were performed using the SPSS (Statistical Package for Social Sciences) version 10.0. The following variables were entered into the model: the number of previous miscarriages, the age of the patient, treatment status, the presence or absence of other autoantibodies (ANA, α-dsDNA) and the ACA test status. Age and number of previous miscarriages were entered as continuous variables whereas the remaining variables were divided into binary categories. The Odds Ratios (ORs) in the logistic regression analysis were calculated as the ratios between the odds for live birth in patients positive for an independent variable and the odds for live birth in patients negative for the variable with or without adjustment of the impact of the other variables. With regard to the continuous variables, the ORs refer to the ratios between the odds for live birth in the group with a higher number of previous miscarriages or age and the group with the number of miscarriages or age just below. Using backward elimination we excluded variables that were not significantly associated with the outcome measure. The relevant two-factor interaction term defined as the product of treatment and ACA positivity was tested in the model. The OR for the interaction variable is the odds for live birth in treated ACA positive patients divided by the odds for live birth in all other patients (untreated ACA positive patients and treated and untreated ACA negative patients).

Testing the squared values of the continuous variables age and number of miscarriages confirmed the adequacy of the model.

Fisher’s exact test and independent samples test were used to investigate for differences between ACA positive and ACA negative patients with regard to late pregnancy complications in those patients who gave birth to a child.

Results
Clinical outcome
Out of 147 patients included in the present study, 81 (55%) gave birth to a live child while 66 suffered another miscarriage. All of the miscarriages were in the first trimester except for five that occurred between gestational weeks 13 and 20. Karyotyping of the miscarriages was successfully carried out in 23 of the 66 abortuses and only one was found to be chromosomally abnormal.

Cut-off values predicted by this study
Figure 1 shows the live birth rates in this study when the patients are divided into decile groups according to the mean (sample I and II) of IgM ACA and IgG ACA values. For IgM ACA values higher than 4.6 MPL-U/ml (separating the 6th and 7th decile groups) and IgG values higher than 12.8 GPL-U/ml (separating the 8th and 9th decile groups), the live birth rates were in general lower than in lower decile groups and below 52% and these values were chosen as the cut-off levels for ACA (ACA-RM values) derived from this prospective study. In order to be designated ACA-RM+, a patient thus had to have IgM ACA-RM ≥ 4.6 MPL-U/ml and/or IgG ACA-RM ≥ 12.8 GPL-U/ml in both samples I and II.

Testing for heterogeneity
Comparing live birth rates in the six ACA-RM+ subgroups revealed no significant heterogeneity ($\chi^2 = 4.9, P = 0.43$, degrees of freedom = 5), neither did the comparison of live birth rates between the six ACA-RM– subgroups ($\chi^2 = 9.4, P = 0.1$, degrees of freedom = 5).

ACA tests
Among all 147 study patients, 60 (41%) were ACA-RM+, 47 (32%) were IgM ACA-RM+, 19 (13%) were IgG ACA-RM+ and 31 (21%) were ACA-NP+. With regard to the samples taken in the ALT trial we have previously reported that ALT therapy does not change ACA concentrations (Christiansen et al., 1992). With regard to the samples in the IVlg trials, the median concentration of IgM ACA in sample I was 4.2 MPL-U/ml and 4.4 MPL-U/ml in the placebo and treatment groups, respectively, and the corresponding concentrations were 4.9 and 5.0 MPL-U/ml for sample II. The median concentration of IgG ACA in sample I was 6.2 GPL-U/ml and 6.0 GPL-U/ml in the placebo and IVlg treatment groups, respectively, and the corresponding concentrations were 6.0 and 8.5 GPL-U/ml in sample II. Neither of the median concentrations was statistically significantly different between the placebo and treatment groups.

Table I shows the distribution of ACA-RM+ and ACA-NP+ patients according to inclusion in either of the two trials and allocation groups. The frequencies of a positive test were not statistically significantly different between patients in the treatment and placebo groups.

In Tables II and III are shown the live birth rates according to ACA status stratified for number of previous miscarriages and age, respectively. There was a decrease in live birth rate with increased number of previous miscarriages and increased age for both ACA-RM+ and ACA-NP+. The live birth rate tended at each stratum of numbers of miscarriages and age to be lower in the ACA positive groups. There was a statistically significant correlation between the number of previous miscarriages and the frequency of ACA positivity in the patients regardless...
For abbreviations: ACA-RM and ACA-NP, see Materials and Methods (Section: Establishment of cut-off levels); Pregnancies, total number of pregnancies in the study.

<table>
<thead>
<tr>
<th>Previous miscarriages</th>
<th>ACA-RM–</th>
<th>ACA-RM+</th>
<th>ACA-NP–</th>
<th>ACA-NP+</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pregnancies*</td>
<td>Live birth rate</td>
<td>Pregnancies*</td>
<td>Live birth rate</td>
</tr>
<tr>
<td>3</td>
<td>37</td>
<td>81%</td>
<td>13</td>
<td>46%</td>
</tr>
<tr>
<td>4</td>
<td>29</td>
<td>62%</td>
<td>25</td>
<td>48%</td>
</tr>
<tr>
<td>≥ 5</td>
<td>21</td>
<td>43%</td>
<td>22</td>
<td>27%</td>
</tr>
<tr>
<td>Total</td>
<td>87</td>
<td>66%</td>
<td>60</td>
<td>40%</td>
</tr>
</tbody>
</table>

For abbreviations: ACA-RM and ACA-NP, see Materials and Methods (Section: Establishment of cut-off levels); Pregnancies, total number of pregnancies in the study.

<table>
<thead>
<tr>
<th>Previous miscarriages</th>
<th>ACA-RM–</th>
<th>ACA-RM+</th>
<th>ACA-NP–</th>
<th>ACA-NP+</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pregnancies*</td>
<td>Live birth rate</td>
<td>Pregnancies*</td>
<td>Live birth rate</td>
</tr>
<tr>
<td>30–34 years</td>
<td>29</td>
<td>62%</td>
<td>24</td>
<td>46%</td>
</tr>
<tr>
<td>35 years</td>
<td>16</td>
<td>56%</td>
<td>15</td>
<td>20%</td>
</tr>
<tr>
<td>Total</td>
<td>87</td>
<td>66%</td>
<td>60</td>
<td>40%</td>
</tr>
</tbody>
</table>

For abbreviations: ACA-RM and ACA-NP, see Materials and Methods (Section: Establishment of cut-off levels); Pregnancies, total number of pregnancies in the study.

The impact of possible prognostic variables (OR) for a live birth in women with recurrent miscarriage (RM) adjusted for the presence of other autoantibodies with OR = 0.36 (0.2–0.7), P = 0.006; OR = 0.66 (0.5–0.9), P = 0.001 and OR = 0.07 (0.01–0.7), P = 0.02 respectively. Accordingly, the chance of a live birth seems to be reduced to 36% if the RM patient is ACA-RM+ compared with ACA-RM−. The results were similar when the status of IgM ACA-RM was entered as the ACA variable. IgG ACA-RM, however, did not have any significant influence on the chance of live birth. The same trend that IgM ACA-NP rather than IgG ACA-NP impacted the chance of live birth was found even though only half the numbers of patients were ACA positive using these cut-off values. The adjusted ORs for these ACA variables are shown in Table V but the steps of the elimination procedures are not shown to save space. The OR for live birth in ACA-RM+ patients who received placebo in the trials was 0.68 with very wide 95% CIs since the calculations were based on only 65 patients and the impact on live birth rate was not statistically significant (Table V).

### ACA and late pregnancy complications

Out of the 147 patients, 81 gave birth to a live child; 36 of these (44%) were ACA-RM+. The frequency of late miscarriage was not statistically significant (Table III).
pregnancy complications in ACA-RM+ and ACA-RM− is displayed in Table VI. No statistical significant differences between the two groups were found.

Discussion
A statistically significant association between ACA and RM has been reported in many case–control studies (Unander et al., 1987; Barbui et al., 1988; Parazzini et al., 1991; Parke et al., 1991) and autoantibodies including ACA can be found in 20% of these patients (Stephenson, 1996). However, some large case–control studies have failed to show a statistical significant association between ACA and RM or sporadic pregnancy losses (Infante-Rivard et al., 1991; Gris et al., 2000). An important proof for ACA being a causal factor for RM would be given by demonstrating a possible negative impact on future pregnancy outcome since a hallmark for a causal factor is that its presence precedes the event that it is causing. We therefore carried out the present prospective study since, to our knowledge, only three prospective studies have previously looked at the impact of ACA on pregnancy outcome in untreated RM patients (Taylor et al., 1990; Melk et al., 1995; Rai et al., 1995b) with the two first studies showing no effect of ACA and the latter study a very large negative effect. In addition, Laskin et al. (1997) reported a high live birth rate (56%) in placebo-treated patients with two or more previous fetal losses, the majority of whom were positive for ACA.

The present study showed that the OR for live birth in repeatedly ACA positive, LAC negative RM patients was 0.36 (0.2–0.7), \( P = 0.006 \) using cut-off values established in the study (ACA-RM values) and adjusting for the impact of confounding variables (Table IV). The results point at IgM ACA as a more sensitive negative predictor of live birth than IgG ACA. Using cut-off values derived from a normal female population of fertile age (ACA-NP values), the OR for live birth in ACA positive patients was borderline significant before adjustment for prognostic variables but became non-significant after this adjustment resulting in an OR of 0.48 (0.2–1.1), \( P = 0.1 \).

The 60 repeatedly ACA-RM+ patients in the study comprise, to our knowledge, the greatest number of non-anticoagulation-treated ACA positive, LAC negative patients with RM followed prospectively into the next pregnancy being twelve times the number of similar patients followed by Rai et al. (1995b). We believe that the number of ACA positive and negative patients is sufficient for making a thorough analysis, adjusting for the possible confounders by logistic regression, and the estimate of the prospective impact of the antibodies is thus the most exact so far published.

Since heparin and aspirin therapy has gained widespread use—and in many centres is considered mandatory for ACA positive patients—no results of pregnancy outcome in new cohorts of non-anticoagulation treated ACA positive RM women is expected in the future. The widespread use of heparin/aspirin therapy is due to the results from two (non-blinded) trials that found a statistically significantly higher live birth rate in APA positive RM patients receiving both heparin and low dose aspirin compared with low dose aspirin alone (Kutteh, 1996; Rai et al., 1997). In the latter trial almost exclusively patients with LAC were included.

In our study, the chance for live birth in ACA positive RM patients (39–40%) is much higher than the 10% live birth rate very often referred to as reflecting the live birth rate in untreated RM patients with APA (Rai et al., 1995b). This prospective study compared 20 RM patients who were positive for APA and none of whom received any therapy—among these only two ( = 10%) gave birth to live children. However, in this study as many as 15 of the patients (75%) were positive for LAC and only five patients were exclusively ACA positive, indicating that the cohort is not typical for the general RM population.

As pointed out in case–control studies (Lockshin et al., 1989; Gris et al., 2000) and laboratory studies (de Laat et al., 2004) there is a difference between patients identified by the presence of LAC and patients identified by the presence of ACA. The prevalence of ACA in the RM population is generally reported to be much higher than the prevalence of LAC (Cowshock et al., 1986; Unander et al., 1987; Lockwood et al., 1989) and the concordance between LAC and ACA has been reported to vary from 36 to 68% for RM patients (Rote et al., 1990). This was confirmed in the present study since in the original patient population before exclusions were undertaken, only five were LAC positive whereas 65 were ACA-RM+ (including the five LAC positive patients). Among the 60 patients who were ACA-RM+ and LAC negative, none had experienced any thrombotic events whereas three out of the five LAC positive patients had a history of venous thrombosis, indicating that our screening for LAC was indeed quite specific with regard to identifying patients with an increased risk of thrombosis.
Only once before has discrimination between LAC and ACA been done in prospective studies of pregnancy outcome. Out et al. (1992) discriminated between ACA and antibodies with LAC activity in their prospective study of patients with autoimmune disease and repeated pregnancy losses, finding that only the presence of LAC and not ACA was predictive of a miscarriage in the next pregnancy. Interestingly, when discrimination is done between LAC and ACA in the prospective study by Rai et al. (1995b)—the live birth rate was 0% in RM patients with LAC and 40% in those with ACA alone—also providing evidence that the presence of LAC has a much greater negative impact than ACA. We therefore find it important, as done in the present study, to evaluate the influence of ACA alone in the absence of LAC.

In prospective studies, adjustment for the influence of confounders, which can affect the main outcome variable, should always be undertaken when the impact of an independent variable is estimated. The importance of adjusting for other prognostic variables when evaluating the real impact of ACA on live birth rate is evidenced in Tables II and III, showing that the frequency of ACA positive patients increases with the number of previous miscarriages—a well-established negative prognostic factor for live birth rate (Cauchi et al., 1995). The number of miscarriages variable is therefore meeting the criteria of being a confounder in this study, since it is significantly associated to both ACA and live birth rate. It cannot be ruled out that the production of ACA may be the result of the previous carriages of a dead fetus in the uterus. A negative impact on pregnancy outcome of non-APA autoantibodies has often been reported (Gleicher and el-Roeiy, 1988; Tulppala et al., 1993; Matzner et al., 1994). Since non-APA autoantibodies are often found associated with ACA we also found it important to control for the presence of these antibodies.

Our logistic regression analysis indicated that when adjustment for the effect of the variables selected to be of importance (Table IV) is carried out, the impact of presence of ACA defined by a normal population-derived cut-off value on pregnancy outcome becomes statistically insignificant. However, no influence of the confounder control on the impact of the patient-derived cut-off value could be seen.

Although few patients in our study were positive for ANA and α-dsDNA, these autoantibodies remained as significant negative predictors of live birth after adjustment. This is in line with Gleicher’s hypothesis of the reproductive autoimmune failure syndrome (Gleicher and el-Roeiy, 1988): that a series of autoantibodies can be found with increased prevalence in RM patients as a sign of a more wide-spread autoimmune response in these patients. It is possible that the presence of ACA in the absence of LAC, together with the presence of other autoantibodies, is a marker for a general tendency to autoreactivity, which is prognostically bad for pregnancy although the autoantibodies, including ACA, might per se be epiphenomena.

Our study is the first prospective study with confounder control in a population of exclusively RM patients. Out et al. (1992) adjusted for confounders in their study in a mixed population of patients with lupus erythematosus or with one or more prior fetal losses and found that a new miscarriage was predicted by the presence of LAC or a history of RM but not the ACA level. Lynch et al. (1994) in a population of healthy pregnant women found a significant association of IgG ACA and pregnancy loss after adjustment for confounders.

There are a number of potential limitations to our study. In the study were included patients who participated in three previously published randomized placebo-controlled trials of immunotherapy with allogeneic lymphocytes or IV Ig and it cannot totally be ruled out that some of the treatments influence pregnancy outcome in ACA positive patients. An analysis limited to the placebo arms of the studies might have been preferable but since it would then be restricted to much fewer patients the estimate of the OR for livebirth in ACA-RM+ patients would have wide 95% confidence limits: 0.2–2.0 (Table V), which is too imprecise to be of clinical value. We believe that patients in the treated groups can be included in the study due to the following arguments: (i) assignment to the various treatments was independent of whether the patients were positive of ACA or not, (ii) neither of the treatments affects the ACA titres (Christiansen et al., 1992; this study), (iii) there was no evidence that the treatments have a selective effect in ACA positive patients since the two-factor interaction term between treatment and ACA-positivity was not statistically significant in the backward elimination procedure in the logistic regression analysis, (iv) tests for heterogeneity did not find the lymphocyte-immunized, IV Ig-treated and the placebo groups significantly different with regard to live birth rates in ACA positive and negative patients. Patient groups in different trials can be combined in meta-analyses if no statistically significant heterogeneity of the trials with regard to the main outcome measure can be demonstrated and we therefore find it justified combining patients receiving different treatments. In addition, any other biases in age, the numbers of previous miscarriages etc. between the different groups were adjusted for in the logistic regression analysis.

The negative impact on pregnancy outcome was in our study primarily confined to the presence of IgM ACA and not IgG ACA, although we admit that there were only few patients in the latter group.

In contrast to this, some studies have pointed towards IgG ACA as having the highest correlation to pregnancy loss (Lynch et al., 1994). The hypothesis behind the claim that IgM ACA is a less sensible predictor of miscarriage is that a high incidence of IgM ACA has been found in the setting of infectious diseases (Unander et al., 1987), however, none of our patients had had infectious disease at the time of the blood samples or in the 3 month period before. The role of the different isotypes of ACA in RM must be elucidated in further prospective studies.

Our study is the first prospective study in RM patients where all patients designated to be ACA+ had repeatedly positive tests. It is recommended that due to the fluctuation of ACA, emphasis should only be put on an ACA test that remains positive in two different blood samples taken with at
least 6 weeks interval (Wilson et al., 1999). The usefulness of this criterion for making ACA studies more homogeneous can be questioned as long as there is no common definition of the cut-off values and no criteria for the tests’ dating in relation to pregnancy. Since ACA are fluctuating, a positive test taken during or immediately before pregnancy must logically be of greater prognostic value than a test taken months before. In the present study, we made efforts to ensure that the ACA tests really were relevant for the next pregnancy since all patients designed as ACA positive were positive during early pregnancy and cut-off values obtained by two different methods were used.

The Varelisa test kit used for detection of ACA fulfilled the requirements for detection of clinically important ACA (Galli et al., 2003): the wells contained β2-GPI, the buffer contained bovine serum albumin and the intra-assay and inter-assay variability coefficients were appropriate (Wong et al., 2004).

The cut-off levels used in the present study based on the actual outcome (ACA-RM values) need confirmation in future studies since they were (post-hoc) selected to be the cut-off values with the highest specificity for pregnancy loss in this study but this may not hold true in another study. Indeed if the ACA-RM cut-off levels are applied on our control population of healthy females of fertile age, 30% would be positive for IgM and/or IgG ACA so it is not obvious which of the two cut-off levels tested in this study is superior. The ACA-NP cut-off values may be the best for differentiating between women who are at risk for developing RM and those who are not, whereas the ACA-RM cut-offs may be best for predicting pregnancy outcome in patients who have already got the RM diagnosis.

Several studies have reported that the risk of fetal growth retardation and pre-eclampsia is increased in ACA positive women (Branch, 1992; Yasuda et al., 1995; Kutteh et al., 1996). In the present study no differences were found concerning pregnancy complications and ACA status (Table VI), however, it must be emphasized that the study was made primarily to assess the impact of ACA on live birth rate and had not enough statistical power to evaluate the frequency of late pregnancy complications.

In conclusion, our study and other prospective studies (Taylor et al., 1990; Melk et al., 1995) suggest that the real impact of ACA on pregnancy outcome in RM in the absence of LAC is less than generally believed. In the present study the most likely chance for live birth in ACA positive patients is 36–48% of the chance of ACA negative patients. However, the 95% confidence intervals indicate that the real chance of live birth could range from as low as 20% to as high as 70% of that of ACA negative patients. Factors responsible for our findings are probably the focus on patients negative for LAC and the adjustment for the influence of other prognostic factors.

We believe that the results stress the urgent need for real placebo-controlled trials of anticoagulation therapy and other forms of therapy in LAC negative, ACA positive patients, taking into account that one of the two heparin/aspirin trials included almost exclusively the minority of patients that were LAC positive (Rai et al., 1997). If these trials are not carried out, dozens of women will daily be exposed to a treatment with potential risks (although low) of osteoporosis, and/or haemorrhage (Ruiz-Irastorza et al., 2001), together with economic costs, which might have less therapeutic effect than previously believed because the spontaneous prognosis in ACA positive, LAC negative RM patients seems to be fair.

References


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